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THE ULTRASTRUCTURAL ORGANIZATION OF THE BLUE-GREEN ALGA MICROCYSTIS AERUGINOSA KUETZ. EMEND. ELENK IN CONNECTION WITH TOXICOGENESIS

by

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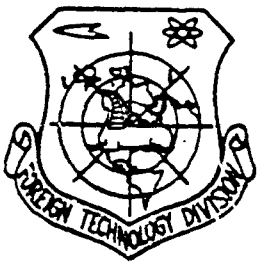

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А а	*А а*	A, a	Р р	*Р р*	R, r
Б б	*Б б*	B, b	С с	*С с*	S, s
В в	*В в*	V, v	Т т	*Т т*	T, t
Г г	*Г г*	G, g	У у	*У у*	U, u
Д д	*Д д*	D, d	Ф ф	*Ф ф*	F, f
Е е	*Е е*	Ye, ye; E, e*	Х х	*Х х*	Kh, kh
Ж ж	*Ж ж*	Zh, zh	Ц ц	*Ц ц*	Ts, ts
З з	*З з*	Z, z	Ч ч	*Ч ч*	Ch, ch
И и	*И и*	I, i	Ш ш	*Ш ш*	Sh, sh
Й й	*Й й*	Y, y	Щ щ	*Щ щ*	Shch, shch
К к	*К к*	K, k	Ъ ъ	*Ъ ъ*	"
Л л	*Л л*	L, l	Ы ы	*Ы ы*	Y, y
М м	*М м*	M, m	Ь ь	*Ь ь*	'
Н н	*Н н*	N, n	Э э	*Э э*	E, e
О о	*О о*	O, o	Ю ю	*Ю ю*	Yu, yu
П п	*П п*	P, p	Я я	*Я я*	Ya, ya

*ye initially, after vowels, and after ъ, ь; e elsewhere.
When written as ё in Russian, transliterate as yё or ё.

RUSSIAN AND ENGLISH TRIGONOMETRIC FUNCTIONS

Russian	English	Russian	English	Russian	English
sin	sin	sh	sinh	arc sh	$\sinh^{-1}$
cos	cos	ch	cosh	arc ch	$\cosh^{-1}$
tg	tan	th	tanh	arc th	$\tanh^{-1}$
ctg	cot	cth	coth	arc cth	$\coth^{-1}$
sec	sec	sch	sech	arc sch	sech^{-1}
cosec	csc	csch	csch	arc csch	csch^{-1}

Russian	English
rot	curl
lg	log

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The Ultrastructural Organization of the Blue-Green Alga Microcystis Aeruginosa Kuetz. Emend. Elenk in Connection With Toxicogenesis

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(Submitted to the editors 5 April 1971)

At the present time the blue-green alga Microcystis aeruginosa Kuetz. emend. Elenk is attracting the attention of many researchers. This is explained by the fact, that the majority of strains of this species is capable of producing a very strong toxin. The water of reservoirs, where blue-green algae abound, mixed with this toxin, becomes toxic. This water is capable of causing specific gastro-intestinal diseases in man and animals (Gorham, 1964).

With the employment of light-optical methods it has been established, that within the limits of the species Microcystis aeruginosa Kuetz. emend. Elenk there are various morphological forms, differing with respect to the structure of colonies and individual cells (Kondrat'yeva, 1968). The ultrastructural organization of this alga, knowledge of which is necessary for understanding the toxin formation process, has

still not been completely studied.

The data on the submicroscopic structure of M. aeruginosa in the existing literature is sparse (Smith and Peat, 1967). The mentioned authors have studied this species for the purpose of detecting gas vacuoles.

Our task was to study the ultrastructural organization of one strain of M. aeruginosa, isolated from the water of the Kremenchug reservoir by workers of the Institute of Hydrobiology of the Academy of Sciences of the UkSSR, where the physiology of this alga is being studied.

At the present time it is not possible to isolate a pure culture of this alga, because during its intensive purification from accompanying bacteria M. aeruginosa undergoes morphological and physiological-biochemical changes (Gorham and Zehnder, 1960).

We raised algae on a Fitzgerald-Chu medium in a Gorham and Zehnder modification with the addition of microelements (Gorham and Zehnder, 1960), at 22-25° and with illumination by daylight bulbs over a period of 8-10 hours per day with an illumination intensity of 2500-4000 lux.

The algae were fixed in the logarithmic growth phase (40-50-th day) with glutaric aldehyde based on an acetate-veronal buffer with a meat-peptone (beef-extract) broth (Solov'yeva, 1970), and then with osmium by the Ryter-Kellenberger method (1958). Ultra-thin sections were obtained on an LKB ultratome, they were contrasted with 5% aqueous uranyl acetate and then by the Reynolds method 91963) for a period of 5 min. The obtained specimens were studied on a Japanese JEM-6C microscope.

The M. aeruginosa (aeroginosa) cells had round or oval shape on the ultra-thin sections (Fig. 1 inset). The external layer, surrounding the entire cell and separating it from the environment, has a non-uniform electron-optical density and thickness. This layer lies close to the external membrane of the cell wall and upon the examination of the alga was not detected under a light microscope. A similar layer,

having a polysaccharide nature, has been described in other Cyanophyta (Drews and Giesbrecht, 1966). Such features are characteristic for the microcapsule of Gram-negative bacteria (Avakyan and his coauthors, 1967).

The cell wall of M. aeroginosa is multilayered (about 550 Å) - it consists of a three-layer external membrane, and internal layer and two intermediate layers.

The external membrane (Fig. 1, b in the insert and 2) has a thickness of from 110 to 120 Å and has sinuous configurations. It consists of three layers - two external osmium-philic each about 50 Å thick, and one internal electron-transparent layer from 10 to 30 Å thick. Beyond the external membrane there is an electron-transparent intermediate layer, which due to bendings has a nonuniform thickness - from 20 to 150 Å. The internal layer of the cell wall is formed from a homogeneous material of high electron-optical density. Over its entire extent it has a uniform thickness of about 120 Å. Subsequently there is a second electron-transparent intermediate layer, which like the first intermediate layer has a nonuniform thickness - from 50 to 150 Å - due to bends in the cytoplasmic membrane, which follows directly after it (see Fig. 2).

The structure of the cell wall in its main features was identical in all the representatives of blue-green algae. There is a similar structure also in the cell wall of certain Gram-negative bacteria, in particular of certain photosynthesizing bacteria (Tomina and Fedorov, 1967; Solov'yeva and Fedenko, 1970), and also of the chemoautotrophs, Thiobacillus thiooxidans and Thiobacillus ferroxidans, investigated by us earlier (Avakyan and Karavayko, 1970). This fact is additional evidence of the evolutionary relationship of Gram-negative bacteria and the blue-green algae.

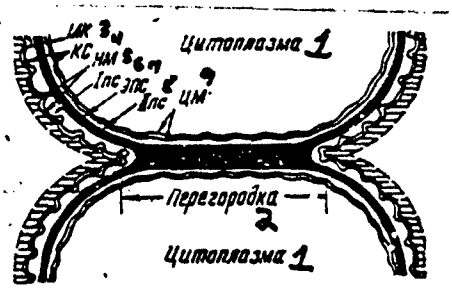

Fig. 2. A diagram of the composition of the surface structures of M. aeroginosa Kuetz. emend. Elenk.
KEY: 1 - Cytoplasm; 2 - Septum (partition); 3 - mk - microcapsule; 4 - ks - cell wall; 5 - nm - external membrane; 6 - Ips - first intermediate layer; 7 - eps - electron-proof layer; 8 - IIps - second intermediate layer; 9 - tsm - cytoplasmic membrane.

The cytoplasmic membrane of the investigated strain is three-layered and about 85 Å thick, it has the appearance of a symmetrical, wavy, two-contour membrane, and it does not lie close to the cell membrane. This type of structure of the cytoplasmic membrane is also typical for Gram-negative bacteria (Avakyan and his coauthors, 1967).

The greater part of the cytoplasm is occupied by the photosynthesizing apparatus - the parachromatophore after Peshkov (1964). The parachromatophore has a lamellar structure and is located mainly along the periphery of the cell. The lamellas are frequently parallel to each other, forming a great number of bends, between which other cellular stuctures are visible - ribosomes, the nucleoid, and various inclusions. The lamellas of M. aeroginosa are three-layered. The intermediate layer has a variable thickness, approximately from 80 to 800 Å, it is limited by 2 electron-proof layers, each 80 -85 Å thick. Thus, it is evident on the ultra-thin sections, that each lamella consists of two photosynthesizing membranes, which do not lie close to each other. A similar structure of the parachromatophore lamellas was detected in representatives of the genus Oscillatoria and Phormidium (Jost, 1965; Drews and Giesbrecht, 1966) in contrast to the paired membranes of Anacystis nidulans and Gloeocapsa alpicola (Ris and Singh, 1961; Echlin, 1964; Allen, 1968).

No membranous structures, similar to the mesosomes of bacteria, endoplasmic or protoendoplasmic reticulum were detected.

Between the lamellas are located granules, similar to ribosomes in shape and size, 110 to 160 Å in diameter, and inclusions spherical in shape, from 930 to 1160 Å in diameter (Fig. 1, a and b). In the cytoplasm there are hexahedral osmium-philic bodies of various size (the smallest size is 1800 x 2500 Å, the largest size is 2900 x 5200 Å), having a fine-granular structure. Similar bodies have been detected in Nostoc muscorum (Reese, 1967).

Moreover, it is possible to see numerous vacuoles, sometimes preserving the shape of a hexahedron (see Fig. 1, a, b). Certain investigators consider, that the vacuoles are formed due to the destruction of

the volutin in the process of preparing the specimen (Levchenko, 1962). Judging from the the ultrastructure of the El Tor cholera vibrio, it is possible to assume, that these vacuoles are connected with toxicogenesis (Avakyan and coauthors, 1972).

The nucleoid of the M. aeroginosa in the sections has the form of individual sites, connected with each other, filled with fibrillar material (see Fig. 1, a).

In spite of the fact, that one of the specific traits of this alga is the presence of gas vacuoles, they were not detected on the ultrathin sections by some of the researchers (Smith and Peat, 1967). We were able to show, that in the cytoplasm of certain, but not all of the cells, there are ordered structures (Fig. 1, c), exremetly similar in structure to gas vacuoles of Oscillatoria rubencens (Jost, 1965) and Trichodesmiummerythraeum (Baalen, 1969). Stacks of parallel membranes, 50 Å thick, are evident in the longitudinal section, the interval between which on the average is 450-700 Å (Fig. 1, e). On the transverse section these structures manifest themselves as honeycomb-like chambers with a cell the size of 650-1000 Å (Fig. 1, c, d). There is evidence, that in other blue-green algae such structures are vacuoles, containing gases - nitrogen, oxygen and argon (Jost and Matile, 1966; Walsby, 1969; cited from Baalen, 1969).

Thus, the structure of M. aeroginosa is similar to the structure of earlier-studied blue-green algae. Characteristic for this strain, raised under the described conditions, is the presence in certain cells of honeycomb-like structures, similar to the gas vacuoles of certain other Cyanophyta. Characteristic also is the presence in the cytoplasm of numerous polyhedral bodies and inclusions of unknown nature.

Conclusions

1. The submicroscopic organization of the studied strain of Microcystis aeroginosa, isolated in the Kremenchug reservoir, is characteristic, as the electron-microscopic study showed, for blue-green algae.

2. Along with the traits, typical of Cyanophyta, features were also detected, characteristic for the given species of algae: the presence in certain cells of honeycomb-like structures, similar to so-called gas vacuoles, the presence in the cytoplasm of polyhedral bodies and inclusions of unknown nature and a sinuous contour of the cytoplasmic membrane, 85 Å thick.

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ULTRASTRUCTURAL ORGANIZATION OF MICROCYSTIS AEROGINOSA KÜTZ. EMEND. ELENK IN CONNECTION WITH TOXICOGENESIS

L. A. Avakyan, O. I. Baulina

The authors studied the submicroscopic organization of Microcystis aeroginosa strain isolated from the waters of Kremenchug water body. This micro-organism has structure characteristic of the Cyanophyta type and some species peculiarities. The structure of the microcapsule and of the cell wall of M. aeroginosa is typical of the majority of fresh-water plants of this species. Cell wall consists of 6 layers; its total thickness is 550 Å. Cytoplasmic membrane (85 Å) has a tortuous outline, which is the peculiarity of the strain under study. The greatest part of the cytoplasm is occupied by parachromatophore; the lamellae forming it consist of two photosynthesizing membranes about 85 Å in thickness with an intermediate layer of inconstant thickness. The cytoplasm contains ribosomes and polyribosomes 110 to 160 Å in diameter, various inclusions, hexahedral bodies. The so called gas vacuoles which were found in the cytoplasm of the majority of the cells serve as one of the species signs. The nucleoid is represented by separate, connected with one another, areas, filled with fibrillar material.

Since many representatives of the species of fresh-water plants under study (particularly a number of M. aeroginosa strains) discharge into the surrounding environment a strong toxin, a study of the mechanism of the toxin formation in the strain described in this work is to be carry out.

Fig. 1. The ultra-fine structure of Microcystis aeroginosa. a - a dividing cell in the logarithmic growth phase. Visible is the cell wall (1 - ks), the cytoplasmic membrane (2 - tsm), the septum (partition) (8 - p), the parachromatophore (3 - pkh), the polyribosomes (4 - r), the hexahedral bodies (7 - sh), the nucleoid (5 - n). Magnification 24,000X; b - cell fragment. Visible is the microcapsule (13 - - mk), the layers of the cell wall - the external membrane (9 - nm), the first intermediate layer (10 - Ips), the electron-proof layer (11 - -eps), the second intermediate layer (12 - IIps), the cytoplasmic membrane (2 - tsm), the photosynthesizing membranes (14 - fk), the spherical inclusions (15 - s), the vacuoles (16 - v). Magnification 40,000X; c - cell fragment, in the cytoplasm of which are gas vacuoles (17 - gv). Magnification 40,000X; d - cell fragment. On the section it is evident, that the gas vacuoles fill almost the entire cytoplasm, masking the other cell structures. Magnification 54,000X; e - a longitudinal section of gas vacuoles (17 - gv). Magnification 90,000X.